REVIEW ARTICLE

Preventing bacterial adhesion on scaffolds for bone tissue engineering

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Abstract: Bone implant infection constitutes a major sanitary concern which is associated to high morbidity and health costs. This manuscript focused on overviewing the main research efforts committed up to date to develop innovative alternatives to conventional treatments, such as those with antibiotics. These strategies mainly rely on chemical modifications of the surface of biomaterials, such as providing it of zwitterionic nature, and tailoring the nanostructure surface of metal implants. These surface modifications have successfully allowed inhibition of bacterial adhesion, which is the first step to implant infection, and preventing long-term biofilm formation compared to pristine materials. These strategies could be easily applied to provide three-dimensional (3D) scaffolds based on bioceramics and metals, of which its manufacture using rapid prototyping techniques was reviewed. This opens the gates for the design and development of advanced 3D scaffolds for bone tissue engineering to prevent bone implant infections.

Keywords: Antibacterial adhesion, biofilm formation, zwitterionic surfaces, nanostructured surfaces, rapid prototyping 3D scaffolds, bone tissue engineering.

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1. Introduction

The infection risk of bone implants is a major clinical concern that could lead to implant failure and subsequent serious postoperative complications of surgical procedures with high morbidity and costs to the national healthcare systems. Bone implant infections are usually caused by bacterial attachment and colonization on the implant surface[1]. Bacterial adhesion and subsequent growth usually results in slime enclosed biofilm formation on the implant surface[2,3]. In fact, it has been estimated that 65–80% of bacterial infections treated by clinicians in the developed world are caused by organisms growing on biofilms[4]. A biofilm is a microbial-derived sessile community consisting of prokaryotic cells permanently attached to a substratum one to each other, embedded in a matrix of extracellular polymeric substances that it had produced[2]. Bacteria forming biofilms are resistant to host defenses and conventional antibacterial therapies such as vaccines and antibiotics that are effective to eliminate infections caused by planktonic bacteria[5]. Therefore, the initial bacterial adhesion to the biomaterial surface becomes critical in infection pathogenesis.

Of late, new approaches have been proposed to

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control and prevent bacterial contamination of implants. One strategy consisted of tailoring the antibacterial properties of the implant surface. Thus, different surface modifications and coating techniques have been proposed, such as direct impregnation with antibiotics, immobilization of bactericidal agents or coating with antimicrobial active metals such as copper and silver, nitric oxide-releasing materials, and TiO_2 films[6]. Nonetheless, whatever antimicrobial strategies used, implants must fulfill the non-fouling requirements or biomacromolecules and dead microorganisms would easily accumulate on the implant surface and hinder the antimicrobial activity of its functional groups[7]. For this reason, great research efforts have been devoted to develop new strategies to modify the surface of biomaterials to provide antibacterial adhesion capability. With the aim of hampering the attachment of microorganism onto surfaces, a widely investigated method consisted of grafting surfaces with hydrophilic polymers, and highlighting polyethylene glycol (PEG) derivatives. Steric repulsion caused by a water hydration layer formed via hydrogen bonding has often been proposed to explain the resistance of hydrophilic surfaces to protein and bacterial adhesion[8,9]. A major concern that limits biological applications of PEG is that this polyether autoxidizes relatively quickly[10], which made PEG coatings having restricted attainment in preventing long-term biofilm formation.

Recently, zwitterionization of biomaterials has emerged as a groundbreaking strategy to confer surfaces of high resistance to nonspecific protein adsorption, bacterial adhesion and/or biofilm formation[9]. Zwitterions are characterized by owning an equal number of both positively and negatively charged groups within a molecule hence maintaining overall electrical neutrality. The non-fouling ability of zwitterionic materials, as in the case of hydrophilic materials, is correlated with a hydration layer on the surface, since a closely bound water layer forms a physical and energetic barrier to avoid bacterial adhesion. Since zwitterionic materials contain both positive and negative charged units, it can bind water molecules even more strongly than hydrophilic materials via electrostatically induced hydration, becoming an important part in affording interfacial bioadhesion resistance[9,11].

On the other hand, it has been demonstrated that surface nanotopography and architecture plays an essential role in bacterial attachment and biofilm formation[12–15]. In fact, Campoccia *et al.*[16] indicated that the use of nanostructured surfaces with inhibited bacterial adhesion could represent a challenging alternative to antibiotics[17–19]. Varied surface modification techniques have been widely used in the fabrication of artificial antibacterial surfaces[20–22]. These surfaces comprised a range of nanotubes and nanoparticle-based surfaces, and nanostructured coatings produced by glancing angle deposition technique by magnetron sputtering (MS-GLAD)[23,24].

The potential of these antibacterial strategies into the bone tissue engineering (BTE) landscape would be essential in manufacturing advanced three-dimensional (3D) scaffolds. The different techniques used in the manufacturing of scaffolds must permit an accurate control of different length scales from nano, micro to macro[25], attending to clinical needs. 3D scaffolds for BTE must fulfill the following requirements[26]: (i) highly interconnected pore networks to allow cell growth, nutrients supply and metabolic waste; (ii) both biocompatible and bioresorbable behavior with tunable degradation and resorption rates to ensure tissue replacement; (iii) appropriate surface chemistry for selective cell attachment, proliferation, and differentiation; and (iv) mechanical properties similar to those of the tissues at the implantation site[26,27].

This review begins with a description of the different recent surface modification strategies aimed at inhibiting bacterial adhesion. Among the diverse approaches, we centered on the chemical modification of biomaterials via zwitterionization, and the modification of metal implants by tailoring its surface nanotopography. In addition, this review focused on the potential application of these antibacterial strategies in BTE. To this aim, the more sophisticated techniques for the fabrication of 3D scaffolds are overviewed.

2. Bone Implant Infections

In this section we overviewed the recent advances developed to date concerning the design and development of zwitterionic surfaces and nanostructured coatings to inhibit bacterial adhesion and biofilm formation onto implantable biomaterials.

2.1 Zwitterionization of Biomaterials

Zwitterionic materials are very promising next-generation biomaterials with a wide variety of potential biomedical applications. Herein, we summarized the methods reported to date to provide metal substrates and bioceramics of zwitterionic nature aimed at designing bacterial anti-adhesive biomaterials.

(1) *Zwitterionization of metal substrates*

Zwiterionic polymers such poly(sulfobetaine methacrylate) (pSBMA) and poly(carboxybetaine methacrylate) (pCBMA), possessing mixed positively and negatively charged functional groups within the same polymer chain and total neutral charge, exhibit ultra-low fouling capabilities, able to inhibit nonspecific protein adsorption, bacterial adhesion and biofilm formation[28–32]. The most widely used method to graft zwitterionic polymers to surfaces is the surface-initiated atom transfer radical polymerization (SI-ATRP)[33]. Among zwitterionic polymers, pSBMA has been grafted to different substrates such as gold[33], glass[34] and poly(tetrafluoroethylene) membranes[35] to attain unfouling surfaces.

Recently, an improved strategy for surface zwitterionization of metallic surfaces, such as commercial pure titanium (pTi)[36] and biomedical grade 316L type stainless steel (SUS 316L)[37], by SI-ATRP of pSBMA has been reported (Figure 1A). Zwitterionization can

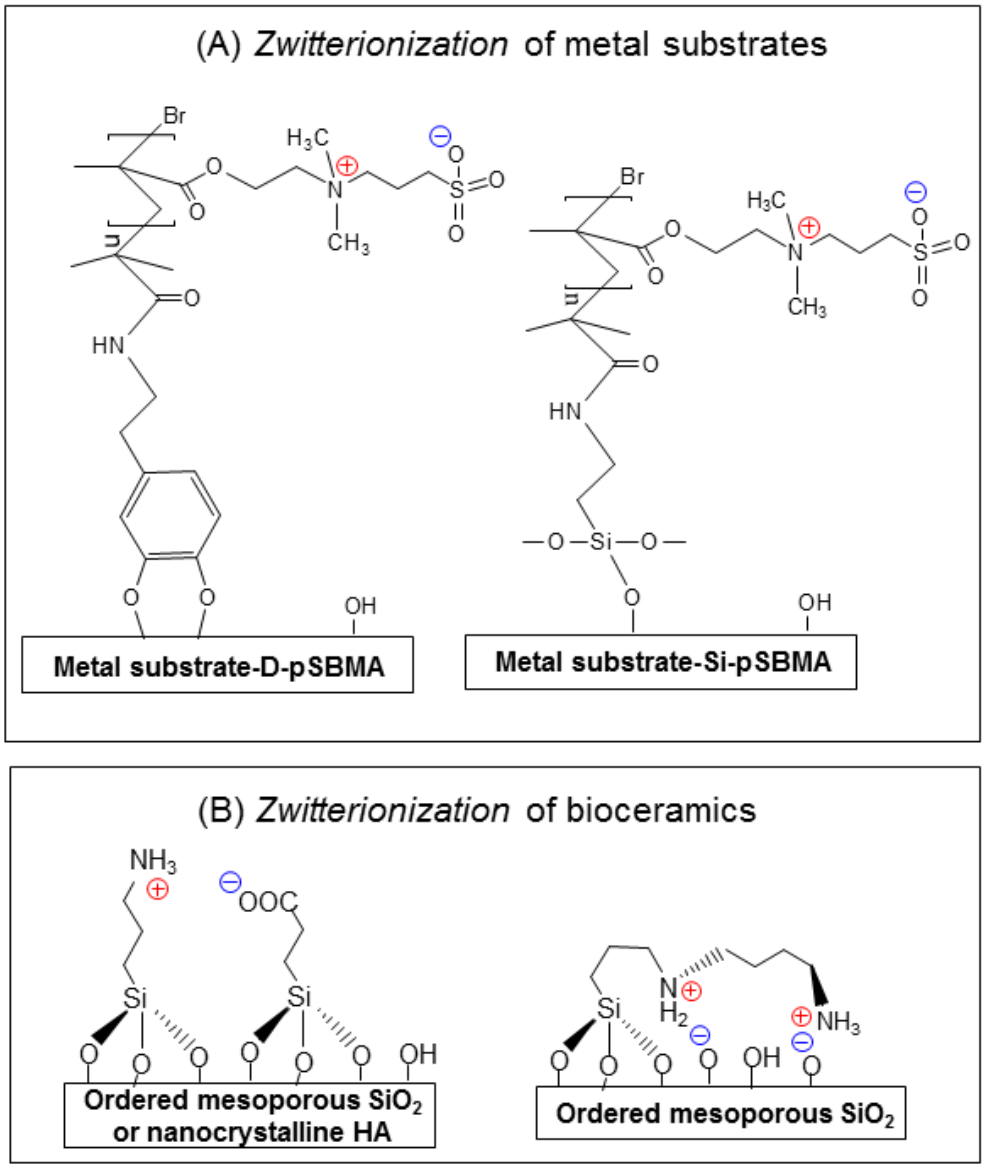

Figure 1. Schematic depiction of the developed strategies to functionalize biomaterials. **(A)** Zwitterionization of metal substrates with poly(carboxybetaine methacrylate) (pCBMA) by covalently bonding dopamine (D) (left) or an organosilane (Si) (right), grafting of an initiator and polymerization of SBMA monomers via surface-initiated atom transfer radical polymerization (SI-ATRP) **(B)** Zwitterionization of bioceramics (ordered mesoporous silica or nanocrystalline hydroxyapatite, HA) by using 3-aminopropyltrimethoxysilane (APTES) and carboxyethylsilanetriol sodium salt (CES) (left) or (N-(2-aminoethyl)-3-aminopropyl-trimethoxysilane) (DAMO) (right).

be divided into four stages[8,9]: (i) treating of the bare metal with ultraviolet (UV) light; (ii) immobilization of either dopamine (D) or an organosilane (Si); (iii) grafting of the initiator, 2-bromoisobutyl bromide (BiBB); and (iv) polymerization of SBMA monomers from the BiBB-tethered surface via ATRP. *In vitro* bacterial adhesion assays were tested using two of the most commonly seen clinical bacteria, *E. coli* and *S. epidermidis*. Bacterial adhesion tests on pTi surfaces indicated that bare metal surface was fully covered by *E. coli* and *S. epidermidis* after 24 hours of assay[36]. However, very few bacteria were attached to SI-ATRP-treated surfaces, reduced to *ca.* 95% relative to uncoated pTi surfaces. This opened up promising expectations in the field of metallic implants.

(2) *Zwitterionization of bioceramics*

Bioceramics are excellent candidates to manufacture bone-like scaffolds[38,39]. It can be designed to release biologically active molecules to repair, maintain, restore or improve bone functions. Different strategies have been developed to provide bioceramics of zwitterionic nature aimed at inhibiting bacterial adhesion and preventing bone implant infections. In this case, inhibition of bacterial colonization must be compatible with adhesion of bone-forming cells to allow osseointegration, which is an essential requisite to warrant a successful implant performance.

Among bioceramics, silica-based ordered mesoporous materials have been broadly proposed for bone tissue regeneration[39–42]. These materials display high surface areas and pore volumes, tailored and narrow pore size distributions, and functionalizable surfaces. These characteristics allow these materials to act as host matrices for a wide range of therapeutic molecules, such as drugs, peptides and small proteins, to be subsequently released in a sustained fashion at the implantation site[43]. Providing the surface of mesoporous matrices of zwitterionic nature to inhibit bacterial adhesion would constitute and add value for the biomedical application of these materials. Thus, the synthesis of zwitterionic SBA-15 type mesoporous material bearing $–NH_3^{\oplus}/–COO^{\ominus}$ groups has been reported (Figure 1B)[44]. This material was synthesized by the co-condensation method using 3-aminopropyltriethoxysilane (APTES) and carboxyethyl silanetriol sodium salt (CES) silanes as $–NH_3^{\oplus}$ and $–COO^{\ominus}$ sources respectively, during the synthesis of SBA-15. The zwitterionic nature of this material in aqueous medium was conserved at pH values around 5.5, as confirmed by determining its isoelectric point by ζ potential measurements. The capability of these materials to inhibit bacterial adhesion was *in vitro* evaluated by simulating severe inflammation/infection conditions, which are usually associated to a decrease in normal pH values[45]. *In vitro* bacterial adhesion assays using *E. coli* indicated that bacterial adhesion in zwitterionic SBA-15 was reduced to *ca.* 93% relative to that for pure silica SBA-15. Moreover, *in vitro* tests with cultured human Saos-2 osteoblasts were performed to investigate the biocompatibility of zwitterionic materials at 7.4, i.e., once normal physiological pH conditions have recovered. The results demonstrated that zwitterionic SBA-15 exhibited good biocompatibility with Saos-2 osteoblasts adhering, proliferating and maintaining its morphological and functional characteristics[45].

Recently, the design and synthesis of a new zwitterionic SBA-15 type bioceramic with dual antibacterial ability has been reported[46]. Its non-fouling capability was derived from the inherent zwitterionic nature of the surface, while the bactericidal capability resulted from its capability to host antibiotics into the mesopores. In this case, zwitterionic SBA-15 mesoporous material was synthetized by using an alkoxysilane bearing primary and secondary amine groups (N-(2-aminoethyl)-3-aminopropyl-trimethoxysilane) (DAMO) based on the co-condensation route. The zwitterionic nature of SBA-15 comes from the $–NH_3^{\oplus}/–SiO^{\ominus}$ and $<NH_2^{\oplus}/–SiO^{\ominus}$ zwitterionic pairs present on the material surface (Figure 1B). *In vitro* adhesion test with *S. aureus* revealed that this zwitterionic bioceramic was capable of decreasing relative bacterial adhesion from 100% (corresponding to pure silica SBA-15) to values lower than 0.1%. This was the first time that such a huge bacterial inhibition capability was found for a mesoporous bioceramic at a physiological pH of 7.4. Moreover, *in vitro* loading and release assays using cephalexin as a model antibiotic demonstrated that zwitterionic SBA-15 can host drugs into its mesopores, releasing it in more than 15 days. This finding unlocks outstanding insights into the design of new bone implants able to play a dual role to treat infections. The zwitterionic nature allowed inhibiting bacterial adhesion, i.e., the first stage of implant infection, whereas release of antibiotics would help eliminate planktonic bacteria in the implant surroundings.

The above-mentioned results opened up promising expectations in the management and prevention of bone implant infections. However, the great scientific challenge is providing bioceramics currently in clini-

cal use with anti-bacterial adhesion properties while preserving its biocompatibility. Nanocrystalline hydroxyapatite (HA) is a calcium phosphate-based bioceramic widely used in dental and orthopedic reconstructive medicine owing to its biocompatibility, bioactivity and osteoconductivity[47]. Although the inherent brittleness of HA limits its use in the restoration of large bone defects, its applications include dental implants, periodontal treatment, alveolar ridge reconstruction and augmentation, orthopedics, maxillofacial surgery, and otolaryngology[47,48]. Thus, HA is commercially available in several physical forms, including powders, particles, granules, dense blocks, self-setting cements, porous 3D scaffolds, implant coatings and composite components. The possibility to provide HA of anti-bacterial adhesion capability would be an added value. The research group of Prof. Vallet-Regí reported the preparation of stoichiometric HA, $Ca_{10}(PO_4)_6(OH)_2$, exhibiting zwitterionic surface capable of inhibiting bacterial adhesion while allowing osteoblast colonization[49]. APTES and CES organosilanes were used to functionalize the surface of HA with $–NH_3^{\oplus}$ and $–COO^{\ominus}$ groups, respectively (Figure 1B). In a first approach, the functionalization process was optimized in HA powders prepared using the controlled crystallization method. Then, the validity of this functionalization method for application in HA substrates shaped in several forms was assessed. For this purpose, HA 3D scaffolds were fabricated by RP technique (see Section 3.1 for further description of this technique) and the resulting 3D-HA scaffolds were functionalized using APTES and CES. *In vitro* bacterial adhesion using *E. coli* under physiological conditions proved that bacterial adhesion in zwitterionic powder HA and 3D-HA decreased 92% and 99% respectively with respect to unmodified HA materials (Figure 2A). The presence of $–NH_3^{\oplus}/–COO^{\ominus}$ zwitterionic pairs onto HA surface accounts for its bacterial anti-adhesive properties. To evaluate the biocompatibility of these HA surfaces, *in vitro* assays were performed using HOS cell cultures. Thus, zwitterionic and pristine HA samples, both as powder and 3D scaffolds were used to carry out the *in vitro* tests. Osteoblastic like-cell spreading was observed in all samples. High magnification scanning electron microscopy (SEM) micrographs showed viable and well-spread cells, which preserved the typical osteoblast morphology (Figure 2B). Regarding cell morphology, there were no differences between zwitterionic and bare HA samples. Moreover, HA-3D scaffolds exhibited different scales of porosity, i.e., channels of *ca.* 800 μm and macropores at 0.01–600 μm range, allowing good cellular internalization with adequate cell anchorage and cell colonization over the entire surface of the scaffolds.

2.2 Development of Nanostructured Surfaces

Albeit a well-established application of nanotechnology in electronic and optical engineering, the use of nanostructured materials in medicine and biology is still at its infancy. In this sense, it had demonstrated the major role of surface nanotopography in bacterial adhesion and biofilm formation[15-17,50]. Different studies using modeled nanostructured surfaces have demonstrated the influence of the nanostructure in the inhibition of bacterial adhesion[18,51,52].

Nature constitutes an unexhausted font of inspiration for scientists and engineers, particularly in biomimetics[13]. Several natural surfaces are able to maintain a contaminant-free status despite the innate abundance of contaminants in the surroundings[53–57]. Most of these surfaces owe its non-fouling characteristics to its superhydrophobic properties, which in turn are largely due to its nanotopography. Many animals (e.g., the wing of cicadae[13], mosquitos[58], etc.) and plants (e.g., lotus (*Nelumbo nucifera*[59])) possess a hierarchical surface with nanotopologic characteristics that significantly increase its hydrophobicity, often to the point of becoming superhydrophobic[60], and repellent to microorganism adhesion. Its antibacterial effects are exclusively due to surface nanostructure and not to surface chemical effect. Several surface modification techniques have been widely used in the construction of artificial antibacterial surfaces based in nanostructured surfaces[22]. These surfaces comprised a range of polymers, nanotubes and nanoparticle-based surfaces in nanoscale, exhibiting bactericidal or anti-biofouling effect.

It should be highlighted that the development of surfaces with simultaneous opposite responses toward osteoblasts and bacterial proliferation would represent a significant achievement in orthopedic implantology[50]. However, there have been very few studies analyzing surfaces that fulfill both conditions[61,62]. The idea of tailoring surfaces with customized and selective responses toward specific cell types (eukaryotic and prokaryotic cells) should be mandatory in the design of biomaterials for TE purposes[63]. In this sense, the key role of surface nanotopography in the stimulation of osteoblast-like cells while reducing bacterial

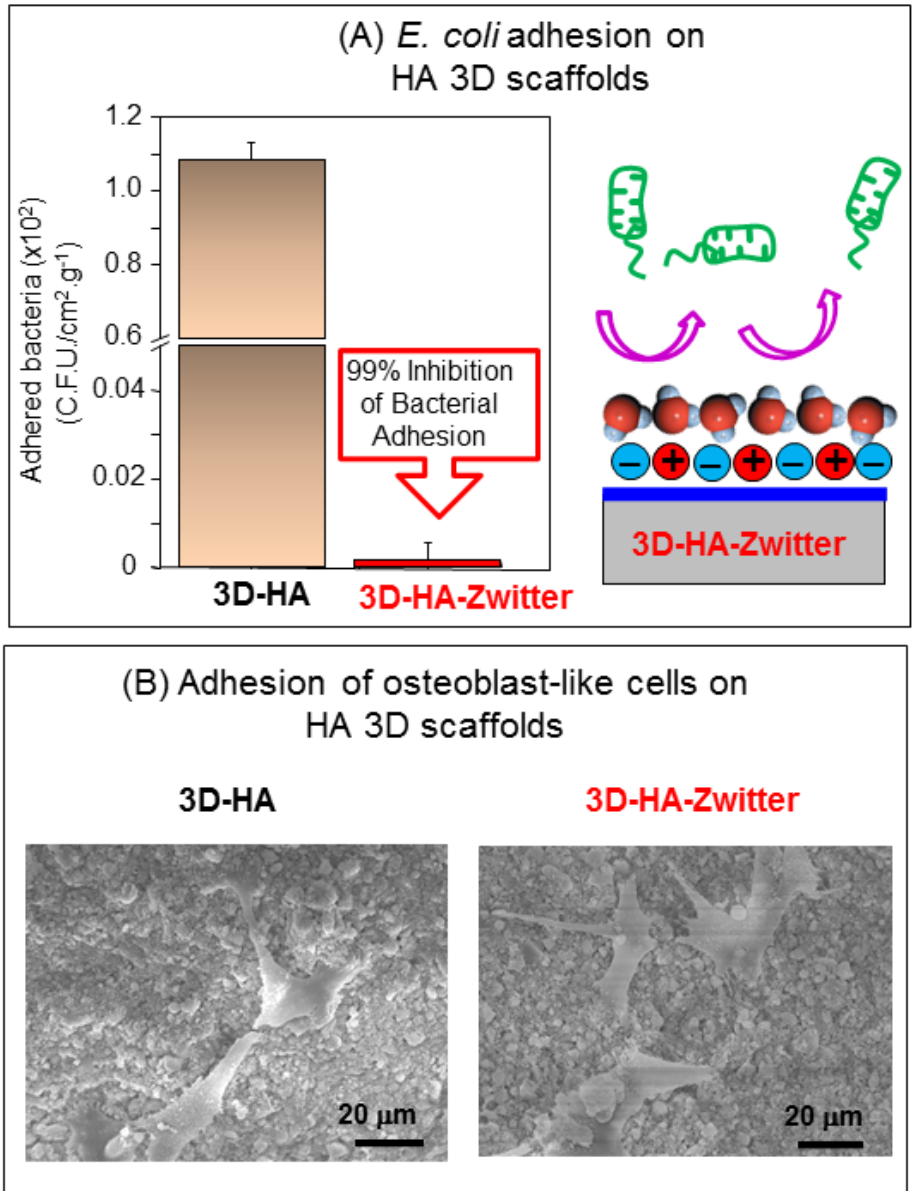

Figure 2. (A) *E. coli* adhesion onto 3D HA scaffolds (3D-HA) before and after being submitted to the zwitterionization process with 3-aminopropyltrimethoxysilane (APTES) and carboxyethyl silanetriol sodium salt (CES) (3D-HA-Zwitter). Schematic representation of the performance of 3D-HA-Zwitter surface during the bacterial adhesion assay was also included. **(B)** SEM micrographs at 1000x magnification of the surface of 3D-HA and 3D-HA-Zwitter scaffolds after 24 hours of cell spreading assay with the HOS osteoblast culture.

adhesion and proliferation can be explained by a mathematical model[63]. Recently, MS-GLAD has been used to produce nanostructured coatings in pure titanium and Ti6Al4V alloy implants[24]. MSGLAD is a powerful technique for producing nanostructured coatings in large areas and with a great variety of morphologies[64]. It is based on exploiting atomic shadowing effects during physical vapor deposition under high vacuum conditions. In this sense, the main processes responsible for the formation of the nanostructures are the atomic self-shadowing mechanism at the surface and the collisional processes of the sputtered atoms in the plasma phase, mediated by the tilt angle of the substrate and the value of the argon background pressure[65].

Figure 3A indicated SEM micrographs corresponding to Ti6Al4V substrates before and after (Nano-Ti6Al4V) MSGLAD processing, displaying different topological surface features. Nano-Ti6Al4V substrate appeared fully coated, with patterns at the

nanoscale consisting of almost vertically aligned nanocolumns with lengths between 250 and 350 nm with a diameter between 40 and 60 nm, separated (from center to center) by 100–200 nm. This nanostructure is very similar to the nanostructure of cicada wings as previously reported[13]. Moreover, this kind of dense, highly packed nanotopography, together with the separation between nanofeatures can lead to a significant

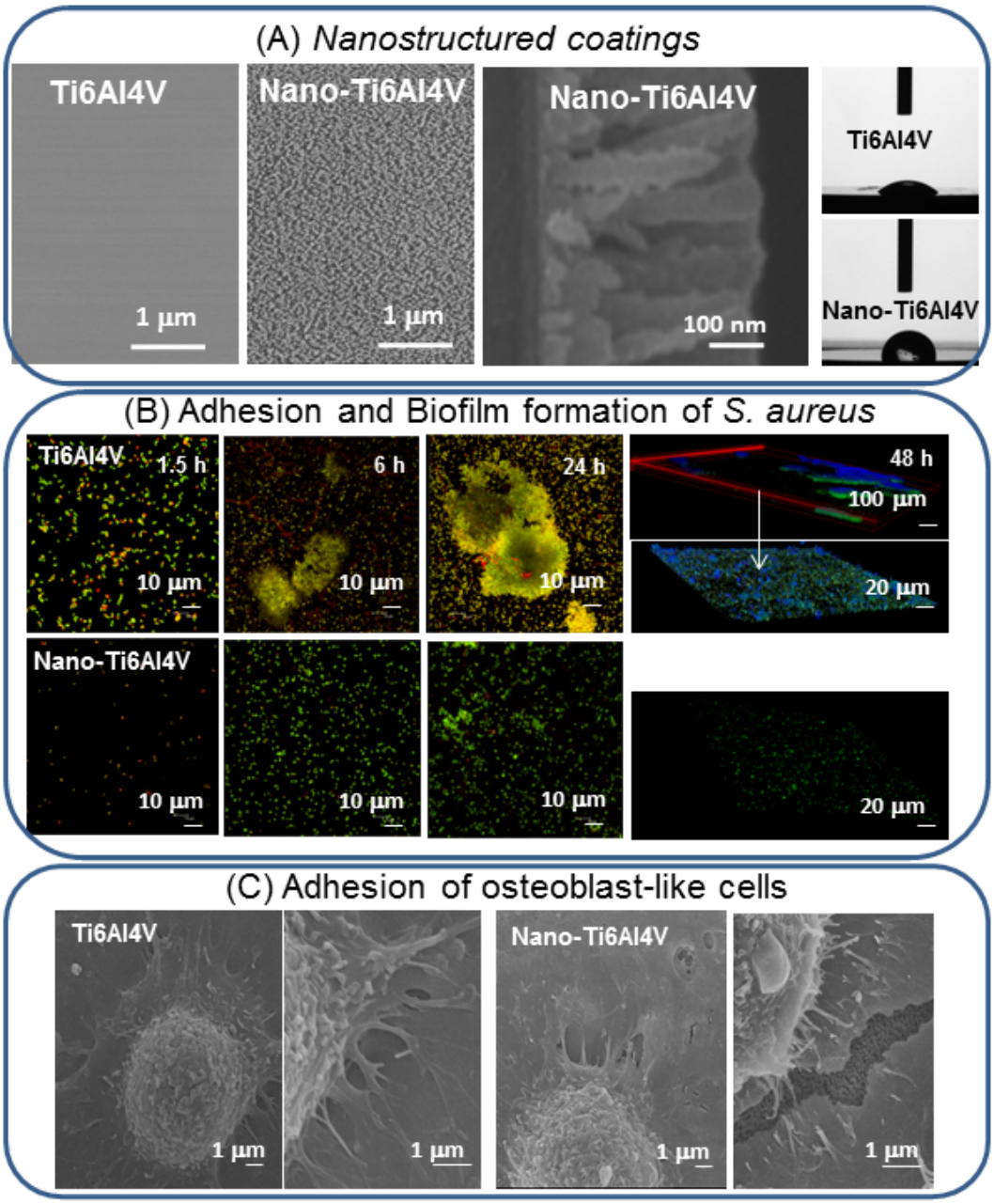

Figure 3. (A) Characterization of nanostructured coating by SEM. Micrographs of Ti6Al4V substrate (smooth surfaces) and Nano-Ti6Al4V surfaces (nanostructured pattering formed by nanocolumns). Evaluation of surface wettability showing the increase of hydrophobicity after coated with nanostructures pattering. **(B)** Images collected by confocal fluorescence microscopy after 1.5, 6 and 24 hours of culture with *S. aureus* on Ti6Al4V and Nano-Ti6Al4V surfaces. Ti6Al4V showed initial bacterial adherence and the subsequent development of a biofilm (6 and 24 hours, arrows). No biofilms were observed in the modified material Nano-Ti6Al4V, and only cells and small conglomerates can be seen. Confocal 3D reconstruction of the Ti6Al4V surface after 48 hours. Extracellular matrix (blue stain) was only observed in Ti6Al4V substrate, on the contrary only live individual bacterial cells was detected on Nano-Ti6Al4V surfaces, with no biofilm formation. **(C)** Osteoblast adhesion after 24 hours on a Ti6Al4V substrate and a Nano-Ti6Al4V sample showing similar behavior.

decrease in wettability due to a ''lotus leaf effect'' on the material surface[56]. To estimate the wettability of the different surface samples, contact angles measurements were measured. The contact angle for the initial Ti6Al4V substrate was 56º whilst that of the Nano-Ti6Al4V was 102º showing a drastic increase in the hydrophobicity for the nanostructured surfaces. The antibacterial effect of the Nano-Ti6Al4V surfaces was evaluated by means of bacterial adhesion experiments and compared with those on medical grade Ti6Al4V substrates. Different *S. aureus* strains from a collection strain and six clinical strains isolated from different patients were used. Results showed that Nano-Ti6Al4V exhibited a notable decrease in *S. aureus* adhesion for both the collection and clinical strains (around 70%) with respect to the untreated Ti6Al4V surfaces.

Concerning biofilm formation, confocal microscopy was used to characterize sequential biofilm formation after different periods (Figure 3B). The presence of a few scattered bacteria on the Nano-Ti6Al4V surface was noted, as well as the absence of biofilm after 24 hours of incubation. Additional confocal microscopy experiments were performed using calcofluor fluorescent stains to stain the extracellular matrix of biofilms after 48 hours (Figure 3B). The confocal 3D images corresponding to biofilm formation demonstrated that non-coated Ti6Al4V substrates clearly show biofilm formation from the blue staining of typical extracellular matrix covering the bacterial colonies. In contrast, blue staining was absent in Nano-Ti6Al4V.

In vitro biocompatibility was assessed by culturing the HOS cell line on the Nano-Ti6Al4V. Results have indicated similar behavior regarding the initial osteoblast adhesion and mitochondrial activity between both surfaces, indicating a good biocompatibility. SEM micrographs after 1 day of culture confirmed that Nano-Ti6Al4V behaved as well as pristine Ti6Al4V with respect to human osteoblasts (Figure 3C). The surfaces in both cases appeared fully covered by cells, exhibiting good adhesion, proliferation and degree of extension. Higher magnification images showed the anchoring elements spread by the cells.

3. Scaffolds for Bone Tissue Engineering

The application of the above-mentioned antibacterial strategies to implants manufactured as 3D scaffolds would represent a step forward in bone tissue engineering (BTE).

Since the emergence of TE in the mid-1980s, a wide variety of shaping methodologies for manufacturing 3D porous scaffolds have been developed. There are many manufacturing methods ranging from the more conventional ones, which lead to randomly interconnected porous scaffolds and that which are principally based on the incorporation of porogen particles[66], use of foam replica technique[67], gel-casting of foams[68], cold isostatic pressing[69], deproteinization of bovine bone[70], particulate leaching[71], freeze-drying[72], gas foaming[73], and a combination of the methods[74]; to more sophisticated technologies based on solid free form (SFF) fabrication such as rapid prototyping (RP). RP techniques allowed accurate control in the macro-microporosity scales and fabricating custom-made implants, which allowed the fabrication of scaffolds both of bioceramic and metallic nature[75]. These techniques constituted a general strategy in which 3D parts are printed layer-by-layer based on a computer-aided-design (CAD) to fabricate 3D interconnected porous scaffolds at a large scale[76,77]. Thus, the scaffold architecture can be adjusted and optimized to attain the adequate mechanical response, accelerate bone regeneration process, and guide bone formation with the anatomic cortical-trabecular structure[78]. Several RP techniques have been used for scaffolds preparation, such as robocasting (RC), selective laser sintering (SLS), selective laser melting (SLM), stereolithography (SLA)[79–82], 3D printing (3DP)[83–85] and fused deposition modeling (FDM)[86,87]. Herein we reviewed the two main RP techniques, namely robocasting (RC) and selective laser based techniques as SLS and SLM, used for the manufacture of bioceramic and metallic scaffolds by itself or in combination with polymers.

3.1 Robocasting (RC)

RC technology also known as direct-printing assembly, is distinctive among these processes because it allowed the building of ceramic scaffolds using water-based inks with minimal organic content (<1wt.%)[88]. Slurries developed from RC must fulfill two important criteria[89]. Firstly, its viscoelastic properties must allow it to flow through a deposition nozzle and then "set" instantaneously so that its shape is preserved as additional layers are deposited or when it span gaps in the underlying structure. Secondly, the suspension must have a high solid volume concentration to reduce shrinkage[90]. The stability of these slurries demands high dispersive forces between particles, where the role of the dispersant is critical[90,91]. The resulting

scaffolds display a high percent of porosity and interconnectivity, easily controllable with enhanced mechanical properties in comparison to conventional scaffold processing (Figure 4A)[92]. Thus, 3D scaffolds based on composites (ceramic-polymer) such as silicon doped hydroxyapatite (SiHA), and mesoporous bioactive glass such as ceramic and polycaprolactone (PCL) and gelatin as polymers have been carried out[93–97]. One of the advantages of RC is the possibility to integrate nanostructural features into micro-macrostructure matrices to fine-tune cellular responses. Thus, the use of sol-gel process combined with RC technique, where the sol can be directly printed before gelation in a one-pot procedure, permits the design of hierarchical 3D meso-macroporous[98]. Hence, the versatility of RC allowed the addition of biodegradable polymers into the slurries avoiding high temperature processing and improving its mechanical properties.

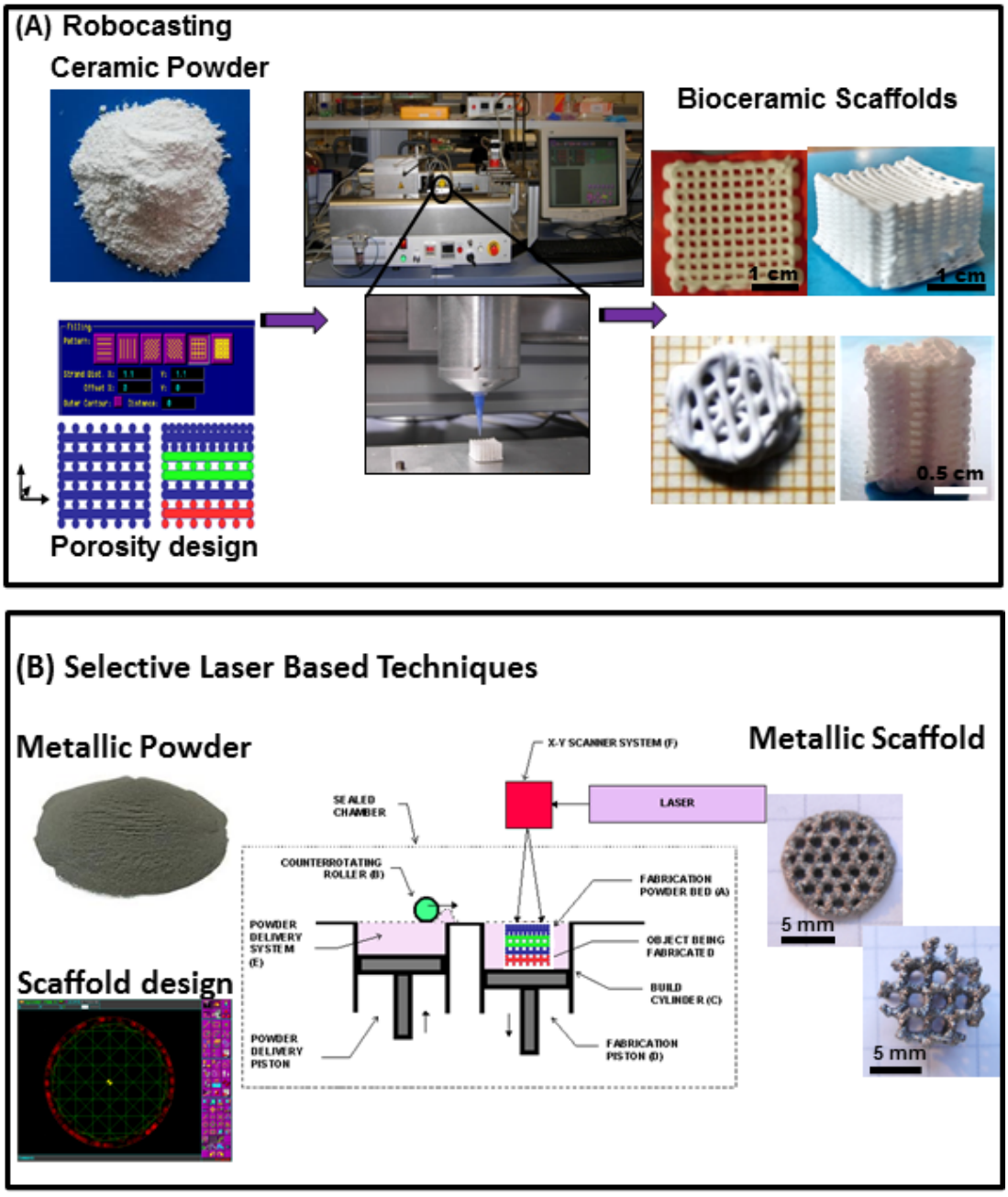

Figure 4. Schematics of two rapid prototyping (RP) methods for the manufacture of 3D scaffolds based in bioceramics and metals via **(A)** robocasting (RC) and **(B)** selective laser based techniques.

This feature permits incorporating biologically active molecules, such as therapeutic drugs and osteoinductive agents, during the manufacture of the scaffold. Thus, 3D scaffolds based in demineralized bone matrix (DBM) containing nanocrystalline silicon doped hydroxyapatite (HA) and PCL have been fabricated[99].

3.2 Selective Laser Based Techniques

Among the most used laser-assisted additive manufacturing are the SLS and SLM techniques[100]. These techniques involved selective use of a laser to build up a model layer by layer from a fine powder bed (Figure 4B). The fine powder particles adhere and sinter when illuminated by a laser beam. Each layer is scanned according to its corresponding cross-section as calculated from the CAD model. The immediate advantage offered by these techniques is that there is no requirement of support structures, since the un-sintered powder provides support during the build and leads to the possibility of obtaining polymeric, ceramic and metallic based-scaffolds[101–102]. Usually, ceramic and polymeric 3D scaffolds are manufactured by the SLS technique, which permits fabricating complex geometries with controllable internal architecture such as those required for BTE applications[47,85,103,104]. *In vivo* evaluations revealed that the 3D ceramic scaffolds were biocompatible and that the macropores were filled by mineralization. For instance, porous calcium phosphate ceramic scaffolds have been fabricated using different weight ratios of tricalcium phosphate (TCP)/HA via SLS. Rapid heating and cooling of SLS were used, which reduced the decomposition of HA due to shorter exposure at high temperature[105].

SLM, which emerged to alleviate some of the issues associated to SLS technique, uses a high-energy laser beam to directly fuse the high-temperature metallic powder layers consecutively deposited as ultra-thin 2D cross-sections. In this sense, SLM has been proven to be beneficial for the manufacture of bone tissue engineering metallic scaffolds and implants by producing very fine and porous structures with mechanical properties similar to those of bulk materials[100,106].

4. Conclusion

Recent scientific advances have permitted the development of novel alternatives to antibiotics treatment for the management and prevention of bone implant infections. The fine tuning of chemical and nano-structural properties of biomaterial surfaces permits providing bioceramic and metallic substrates antibacterial properties by notably reducing bacterial adhesion and biofilm formation compared to bare substrates. The application of these approaches to 3D scaffolds augur promising opportunities in the field of bone tissue engineering.

Conflict of Interest and Funding

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References

1. Nablo B J, Rothrock A R and Schoenfisch M H, 2005, Nitric oxide-releasing sol-gels as antibacterial coatings for orthopedic implants. *Biomaterials*, vol.26(8): 917–924. http://dx.doi.org/10.1016/j.biomaterials.2004.03.031
2. Costerton J W, Montanaro L and Arciola C R, 2005, Biofilm in implant infections: its production and regulation. *International Journal of Artificial Organs*, vol.28(11): 1062–1068.
3. Bjarnsholt T, 2013, The role of bacterial biofilms in chronic infections. *Acta Pathologica, Microbiologica et Immunologica Scandinavica*, vol.121(136): 1–51. http://dx.doi.org/10.1111/apm.12099
4. Lebeaux D, Chauhan A, Rendueles O, *et al.*, 2013, From in vitro to *in vivo* models of bacterial biofilm-related infections. *Pathogens*, vol.2(2): 288–356. http://dx.doi.org/10.3390/pathogens2020288
5. Davies D, 2003, Understanding biofilm resistance to antibacterial agents. *Nature Reviews Drug Discovery*, vol.2(2): 114–122. http://dx.doi.org/10.1038/nrd1008
6. Simchi A, Tamji E, Pishbin F, *et al.*, 2011, Recent progress in inorganic and composite coatings with bactericidal capability for orthopaedic applications. *Nanomedicine: Nanotechnology, Biology and Medicine*, vol.7(1): 22–39. http://dx.doi.org/10.1016/j.nano.2010.10.005
7. Klibanov A M, 2007, Permanently microbicidal materials coatings. *Journal of Materials Chemistry*, vol.17(24): 2479–2482. http://dx.doi.org/10.1039/B702079A
8. Zheng J, Li L, Tsao H K, *et al.*, 2005, Strong repulsive forces between protein and oligo(ethylene glycol) self-assembled monolayers: a molecular simulation study. *Biophysical Journal*, vol.89(1): 158–166.

http://dx.doi.org/10.1529/biophysj.105.059428

9. Chen S, Li L, Zhao, *et al.*, 2010, Surface hydration: Principles and applications toward low-fouling/non-fouling biomaterials. *Polymer*, vol.51(23): 5283–5293. http://dx.doi.org/10.1016/j.polymer.2010.08.022
10. Ostuni E, Chapman R G, Holmlin R E, *et al.*, 2001, A survey of structure-property relationships of surfaces that resist the adsorption of protein. *Langmuir*, vol.17(18): 5605–5020. http://dx.doi.org/10.1021/la010384m
11. Tanaka M, Sato K, Kitakami E, *et al.*, 2015, Design of biocompatible and biodegradable polymers based on intermediate water concept. *Polymer Journal*, vol.47: 114–121. http://dx.doi.org/10.1038/pj.2014.129
12. Chung K K, Schumacher J F, Sampson E M, *et al.*, 2007, Impact of engineered surface microtopography on biofilm formation of *Staphylococcus aureus*. *Biointerphases*, vol.2(2): 89–94. http://dx.doi.org/10.1116/1.2751405
13. Ivanova E P, Hasan J, Webb H K, *et al.*, 2012, Natural bactericidal surfaces: Mechanical rupture of *Pseudomonas aeruginosa* by cicada wings. *Small*, vol.8(16): 2489–2494. http://dx.doi.org/10.1002/smll.201200528
14. Bazaka, K, Crawford R J and Ivanova E P, 2011, Do bacteria differentiate between degrees of nanoscale surface roughness? *Biotechnology Journal*, vol.6(9): 1103–1114. http://dx.doi.org/10.1002/biot.201100027
15. Truonga V K, Lapovok R, Estrin Y S, *et al.*, 2010, The influence of nano-scale surface roughness on bacterial adhesion to ultrafine-grained titanium. *Biomaterials*, vol.31(13): 3674–3683. http://dx.doi.org/10.1016/j.biomaterials.2010.01.071
16. Campoccia D, Montanaro L and Arciola C R, 2013, A review of the biomaterials technologies for infection-resistant surfaces. *Biomaterials*, vol.34(34): 8533–5854. http://dx.doi.org/10.1016/j.biomaterials.2013.07.089
17. Puckett S D, Taylor E, Raimondo T, *et al.*, 2010, The relationship between the nanostructure of titanium surfaces and bacterial attachment. *Biomaterials*, vol.31(4): 706–713. http://dx.doi.org/10.1016/j.biomaterials.2009.09.081
18. Díaz C, Schilardi P L, Salvarezza R C, *et al.*, 2007, Nano/microscale order affects the early stages of biofilm formation on metal surface. *Langmuir*, vol.23(22): 11206–11210. http://dx.doi.org/10.1021/la700650q
19. Jahed Z, Lin P, Seo B B, *et al.*, 2014, Responses of *Staphylococcus aureus* bacterial cells to nanocrystalline nickel nanostructures. *Biomaterials*, vol.35(14): 4249–4254. http://dx.doi.org/10.1016/j.biomaterials.2014.01.080
20. Tiraferri A, Vecitis C D and Elimelech M, 2011, Covalent binding of single-walled carbon nanotubes to polyamide membranes for antimicrobial surface properties. *ACS Applied Materials and Interfaces*, vol.3(8): 2869–2877. http://dx.doi.org/10.1021/am200536p
21. Knetsch M L W and Koole L H, 2011, New strategies in the development of antimicrobial coatings: The example of increasing usage of silver and silver nanoparticles. *Polymers*, vol.3(1): 340–366. http://dx.doi.org/10.3390/polym3010340
22. Kelly P J, Lia H, Whitehead K A, *et al*., 2009, A study of the antimicrobial and tribological properties of TiN/Ag nanocomposite coatings. *Surface and Coatings Technology*, vol.204(6–7): 1137–1140. http://dx.doi.org/10.1016/j.surfcoat.2009.05.012
23. Sengstock C, Lopian M, Motemani Y, *et al.*, 2014, Structure-related antibacterial activity of a titanium nanostructured surface fabricated by glancing angle sputter deposition. *Nanotechnology*, vol.25(19): 195101–195702. http://dx.doi.org/10.1088/0957-4484/25/19/195101
24. Izquierdo-Barba I, García-Martín J M, Álvarez R, *et al.*, 2015, Nanocolumnar coatings with selective behavior towards osteoblast and *Staphylococcus aureus* proliferation. *Acta Biomaterialia*, vol.15: 20–28. http://dx.doi.org/10.1016/j.actbio.2014.12.023
25. Anselme K, 2000, Osteoblast adhesion on biomaterials. *Biomaterials*, vol.21(7): 667–681. http://dx.doi.org/10.1016/S0142-9612(99)00242-2
26. Hutmacher D W, 2000, Scaffolds in tissue engineering bone and cartilage. *Biomaterials*, vol.21(24): 2529–2543. http://dx.doi.org/10.1016/S0142-9612(00)00121-6
27. Hollister S J, 2009, Scaffold design and manufacturing: from concept to clinic. *Advanced Materials*, vol. 21(32–33), 3330–3342. http://dx.doi.org/10.1002/adma.200802977
28. Cheng G, Zhang Z, Chen S, *et al.*, 2007, Inhibition of bacterial adhesion and biofilm formation on zwitterionic surfaces. *Biomaterials*, vol.28(29): 4192–4199. http://dx.doi.org/10.1016/j.biomaterials.2007.05.041
29. Cheng G, Xue H, Zhang Z, *et al.*, 2008, A switchable biocompatible polymer surface with self-sterilizing and nonfouling capabilities. *Angewandte Chemie International Edition*, vol.120(46): 8963–8966. http://dx.doi.org/10.1002/ange.200803570
30. Cheng G, Li G, Xue H, *et al.*, 2009, Zwitterionic carboxybetaine polymer surfaces and their resistance to long-term biofilm formation. *Biomaterials*, vol.30(28): 5234–5240. http://dx.doi.org/10.1016/j.biomaterials.2009.05.058
31. Jiang S and Cao Z, 2010, Ultralow-fouling, functiona-

lizable, and hydrolyzable zwitterionic materials and their derivatives for biological applications. *Advanced Materials*, vol.22(9): 920–932. http://dx.doi.org/10.1002/adma.200901407

32. Lalani R and Liu L, 2012, Electrospun zwitterionic poly(sulfobetaine methacrylate) for nonadherent, superabsorbent, and antimicrobial wound dressing applications. *Biomacromolecules*, vol.13(6): 1853–1863. http://dx.doi.org/10.1021/bm300345e
33. Zhang Z, Chen S, Chang Y, *et al*., 2006, Surface grafted sulfobetaine polymers via atom transfer radical polymerization as superlow fouling coatings. *Journal of Physical Chemistry B*, vol.110(22): 10799–10804. http://dx.doi.org/10.1021/jp057266i
34. Zhang Z, Chao T, Chen S, *et al.*, 2006, Superlow fouling sulfobetaine and carboxybetaine polymers on glass slides. *Langmuir*, vol.22(24): 10072–10077. http://dx.doi.org/10.1021/la062175d
35. Liu Y L, Han C C, Wei T-C, *et al*., 2010, Surface-initiated atom transfer radical polymerization from porous poly(tetrafluoroethylene) membranes using the C-F groups as initiators. *Journal of Polymer Science: Part A: Polymer Chemistry*, vol.48(10): 2076–2083. http://dx.doi.org/10.1002/pola.23975
36. Yu B Y, Zheng J, Chang Y, *et al.*, 2014, Surface zwitterionization of titanium for a general bio-inert control of plasma proteins, blood cells, tissue cells, and bacteria. L*angmuir*, vol.30(25): 7502–7512. http://dx.doi.org/10.1021/la500917s
37. Sin M C, Sun Y M and Chang Y, 2014, Zwitterionic-based stainless steel with well-defined polysulfobetaine brushes for general bioadhesive control. *ACS Applied Materials and Interfaces*, vol.6(2): 861–873. http://dx.doi.org/10.1021/am4041256
38. Vallet-Regí M and Ruiz-Hernández E, 2011, Bioceramics: from bone regeneration to cancer nanomedicine. *Advanced Materials*, vol.23(44): 5177–5218. http://dx.doi.org/10.1002/adma.201101586
39. Vallet-Regí M, 2014, *Bio-ceramics with clinical applications*, John Wiley & Sons Ltd, Chichester, United Kingdom. http://dx.doi.org/10.1002/9781118406748
40. Vallet-Regí M, 2006, Ordered mesoporous materials in the context of drug delivery systems and bone tissue engineering. *Chemistry–A European Journal*, vol.12(23): 5934–5943. http://dx.doi.org/10.1002/chem.200600226
41. Vallet-Regí M, Colilla M and González B, 2011, Medical applications of organic-inorganic hybrid materials within the field of silica-based bioceramics. *Chemical Society Reviews*, vol.40(2): 596–607. http://dx.doi.org/10.1039/C0CS00025F
42. Vallet-Regí M, Izquierdo-Barba I and Colilla M, 2012, Structure and functionalization of mesoporous bioceramics for bone tissue regeneration and local drug delivery. *Philosophical Transactions of the Royal Society of Chemistry A*, vol.370(1963): 1400–1421. http://dx.doi.org/10.1098/rsta.2011.0258
43. Vallet-Regí M, Balas F and Arcos D, 2007, Mesoporous materials for drug delivery. *Angewandte Chemie International Edition*, vol.46(40): 7548–7558. http://dx.doi.org/10.1002/anie.200604488
44. Colilla M, Izquierdo-Barba I, Sánchez-Salcedo S, *et al.*, 2010, Synthesis and characterization of zwitterionic SBA-15 nanostructured materials. *Chemistry of Materials*, vol.22(23): 6459–6466. http://dx.doi.org/10.1021/cm102827y
45. Izquierdo-Barba I, Sánchez-Salcedo S, Colilla M, *et al.*, 2011, Inhibition of bacterial adhesion on biocompatible zwitterionic SBA-15 mesoporous materials. *Acta Biomaterialia*, vol.7(7): 2977–2985. http://dx.doi.org/10.1016/j.actbio.2011.03.005
46. Colilla M, Martínez-Carmona M, Sanchez-Salcedo S, *et al.*, 2014, A novel zwitterionic bioceramic with dual antibacterial capability. *Journal of Materials Chemistry B*, vol.2(34): 5639–5651. http://dx.doi.org/10.1039/C4TB00690A
47. Vallet-Regí M and Navarrete D A, 2015, *Nanoceramics in clinical use: From materials to applications*. 2nd ed., Royal Society of Chemistry, Cambridge, United Kingdom. http://dx.doi.org/10.1039/9781782622550
48. Dorozhkin S V, 2010, Bioceramics of calcium orthophosphates. *Biomaterials*, vol.31(7): 1465–1485. http://dx.doi.org/10.1016/j.biomaterials.2009.11.050
49. Sánchez-Salcedo S, Colilla M, Izquierdo-Barba I, *et al.*, 2013, Design and preparation of biocompatible zwitterionic hydroxyapatite. *Journal of Materials Chemistry B*, vol.1(11): 1595–1606. http://dx.doi.org/10.1039/C3TB00122A
50. Anselme, K, Davidson P, Popa A M, *et al.*, 2010, The interaction of cells and bacteria with surfaces structured at the nanometre scale. *Acta Biomaterialia*, vol.6(10): 3824–3846. http://dx.doi.org/10.1016/j.actbio.2010.04.001
51. Whitehead K A, Colligon J and Verran J, 2005, Retention of microbial cells in substratum surface features of micrometer and sub-micrometer dimensions. *Colloids Surfaces B: Biointerfaces*, vol.41(2–3): 129–138. http://dx.doi.org/10.1016/j.colsurfb.2004.11.010
52. Campoccia D, Montanaro L, Agheli H, *et al.*, 2006, Study of *Staphylococcus aureus* adhesion on a novel nanostructured surface by chemiluminometry. *International Journal of Artificial Organs*, vol.29(6): 622–629.
53. Marmur A, 2004, The Lotus effect: Superhydrophobici-

ty and metastability. *Langmuir*, vol.20(9): 3517–3519. http://dx.doi.org/10.1021/la036369u

54. Su Y, B Ji and Hwang K C, 2010, Nature's design of hierarchical superhydrophobic surfaces of a water strider for low adhesion and low-energy dissipation. *Langmuir*, vol.26(24): 18926–18937. http://dx.doi.org/10.1021/la103442b
55. Bhushan B and Jung Y C, 2011, Natural and biomimetic artificial surfaces for superhydrophobicity, self-cleaning, low adhesion, and drag reduction. *Progress in Materials Science*, vol.56(1): 1–108. http://dx.doi.org/10.1016/j.pmatsci.2010.04.003
56. Guo Z, Liu W and Su B L, 2011, Superhydrophobic surfaces: From natural to biomimetic to functional. *Journal of Colloid and Interface Science*, vol.353(2): 335–355. http://dx.doi.org/10.1016/j.jcis.2010.08.047
57. Webb H K, Hasan J, Truong V K, *et al*., 2011, Nature inspired structured surfaces for biomedical applications. *Current Medicinal Chemistry*, vol.18(22): 3367–3375. http://dx.doi.org/10.2174/092986711796504673
58. Gao X, Yan X, Yao X, *et al*., 2007, The dry-style antifogging properties of mosquito compound eyes and artificial analogues prepared by soft lithography. *Advanced Materials*, vol.19(17): 2213–2217. http://dx.doi.org/10.1002/adma.200601946
59. Bhushan B, Jung Y C, Niemitz A, *et al*., 2009, Lotus-like biomimetic hierarchical structures developed by the self-assembly of tubular plant waxes. *Langmuir*, vol.25(3): 1659–1666. http://dx.doi.org/10.1021/la802491k
60. Koch K, Bhushan B, Yong C J, *et al*., 2009, Fabrication of artificial lotus leaves and significance of hierarchical structure for superhydrophobicity and low adhesion. *Soft Matter*, vol.5(7): 1386–1393. http://dx.doi.org/10.1039/B818940D
61. Ploux L, Anselme K, Dirani A, *et al*., 2009, Opposite responses of cells and bacteria to micro/nanopatterned surfaces prepared by pulsed plasma polymerization and UV-irradiation. *Langmuir*, vol.25(14): 8161–8169. http://dx.doi.org/10.1021/la900457f
62. Mei S, Wang H, Wang W, *et al*., 2014, Antibacterial effects and biocompatibility of titanium surfaces with graded silver incorporation in titania nanotubes. *Biomaterials*, vol.35(14): 4255–4265. http://dx.doi.org/10.1016/j.biomaterials.2014.02.005
63. Decuzzi P and Ferrari M, 2010, Modulating cellular adhesion through nanotopography. *Biomaterials*, vol.31(1): 173–179. http://dx.doi.org/10.1016/j.biomaterials.2009.09.018
64. Alvarez R, García-Martín J M, Macías-Montero M, *et al*., 2013, Growth regimes of porous gold thin films deposited by magnetron sputtering at oblique incidence: From compact to columnar microstructures. *Nanotechnology*, vol.24(4): 045604. http://dx.doi.org/10.1088/0957-4484/24/4/045604
65. García-Martín J M, Álvarez R, Romero-Gómez P, *et al*., 2010, Tilt angle control of nanocolumns grown by glancing angle sputtering at variable argon pressures. *Applied Physics Letters*, vol.97(17): 173103. http://dx.doi.org/10.1063/1.3506502
66. Liu D M, 1996, Fabrication and characterization of porous hydroxyapatite granules. *Biomaterials*, vol.17(20): 1955–1957. http://dx.doi.org/10.1016/0142-9612(95)00301-0
67. Padilla S, Román J and Vallet-Regí M, 2002, Synthesis of porous hydroxyapatite by combination of gelcasting and foams burn out methods. *Journal Materials Science: Materials in Medicine*. vol.13(12): 1193–1197. http://dx.doi.org/10.1023/A:1021162626006
68. Padilla S, Sánchez-Salcedo S and Vallet-Regí M, 2007, Bioactive glass as precursor of designed-architecture scaffolds for tissue engineering. *Journal Biomedical Materials Research*, vol.81(1): 224–232. http://dx.doi.org/10.1002/jbm.a.30934
69. Slosarczyk A J, 1999, Porous hydroxyapatite ceramics. *Journal Materials Science: Materials in Medicine*, vol.18(14): 1163–1165. http://dx.doi.org/10.1023/A:1006677806537
70. Al Ruhaimi K A, 2001, Bone graft substitutes: Acomparative qualitative histologic review of current osteoconductive grafting materials. *International Journal of Oral & Maxillofacial Implants*, vol.16(1): 105–114.
71. Sánchez-Salcedo S, Balas F, Izquierdo-Barba I, *et al*., 2009, *In vitro* structural changes in porous HA/beta-TCP scaffolds under simulated body fluid. *Acta Biomaterialia*, vol.5(7): 2738–2751. http://dx.doi.org/10.1016/j.actbio.2009.03.025
72. Deville S, Saiz E, Nalla R K, *et al*., 2006, Freezing as a path to build complex composites, *Science*, vol.311(5760): 515–518. http://dx.doi.org/10.1126/science.1120937
73. Locs J, Zalite V, Berzina-Cimdina L, *et al*., 2013, Ammonium hydrogen carbonate provided viscous slurry foaming — a novel technology for the preparation of porous ceramics. *Journal of the European Ceramic Society*, vol.33(15–16): 3437–3443. http://dx.doi.org/10.1016/j.jeurceramsoc.2013.06.010
74. Sánchez-Salcedo S, Werner J and Vallet-Regí M, 2008, Hierarchical pore structure of calcium phosphate scaffolds by combination of the gel casting and multiple tape casting methods. *Acta Biomaterialia*, vol.4: 913–922. http://dx.doi.org/10.1016/j.actbio.2008.02.005
75. Hutmacher D W, Sittinger M and Risbud M V, 2004,

Scaffold-based tissue engineering: Rationale for computer-aided design and solid free-form fabrication systems. *Trends in Biology*, vol.22(7): 354–362. http://dx.doi.org/10.1016/j.tibtech.2004.05.005

76. Hutmacher D W, 2001, Scaffold design and fabrication technologies for engineering tissues — state of the art and future perspectives. *Journal Biomaterials Science Polymer Edition*, vol.12(1): 107–124. http://dx.doi.org/10.1163/156856201744489
77. Leong K F, Cheah C M and Chua C K, 2003, Solid freeform fabrication of three-dimensional scaffolds for engineering replacement tissues and organs. *Biomaterials*, vol.24(13): 2363–2378. http://dx.doi.org/10.1016/S0142-9612(03)00030-9
78. Fu Q, Saiz E, Rahaman M N, *et al.*, 2011, Bioactive glass scaffolds for bone tissue engineering: State of the art and future perspectives. *Materials Science and Engineering C*, vol.31(7): 1245–1256. http://dx.doi.org/10.1016/j.msec.2011.04.022
79. Coward T J, Watson R M and Wilkinson I C, 1999, Fabrication of a wax ear by rapid-process modeling using stereolithography. *International Journal of Prosthodontics*, vol.12(1): 20–27.
80. Sánchez-Salcedo S, Nieto A and Vallet-Regí M, 2008, Hydroxyapatite/β-tricalciumphosphate/agarose macroporous scaffolds for bone tissue engineering. *Chemical Engineering Journal*, vol.137(1): 62–71. http://dx.doi.org/10.1016/j.cej.2007.09.011
81. Padilla P, Sánchez-Salcedo S and Vallet-Regí M, 2007, Bioactive glass as precursor of designed-architecture scaffolds for tissue engineering. *Journal of Biomedical Materials Research*, vol.81A(1): 224–232. http://dx.doi.org/10.1002/jbm.a.30934
82. Ryan G E, Pandit A S and Apatsidis D P, 2008, Porous titanium scaffolds fabricated using a rapid prototyping and powder metallurgy technique. *Biomaterials*, vol.29(27): 3625–3635. http://dx.doi.org/10.1016/j.biomaterials.2008.05.032
83. Giordano R A, Wu B M, Borland S W, *et al.*, 1996, Mechanical properties of dense polylactic acid structures fabricated by three dimensional printing. *Journal of Biomaterials Science, Polymer Edition*, vol.8(1): 63–75. http://dx.doi.org/10.1163/156856297X00588
84. Lopez-Heredia M A, Sohier J, Gaillard C, *et al.*, 2008, Rapid prototyped porous titanium coated with calcium phosphate as a scaffold for bone tissue engineering. *Biomaterials*, vol. 29(17): 2608–2615. http://dx.doi.org/10.1016/j.biomaterials.2008.02.021
85. Wiria F E, Shyan J Y M, Lim P N, *et al*., 2010, Printing of titanium implant prototype. *Materials and Design*, vol.31(1): S101–S105. http://dx.doi.org/10.1016/j.matdes.2009.12.050
86. Zein I, Hutmacher D W, Tan K C, *et al.*, 2002, Fused deposition modeling of novel scaffold architectures for tissue engineering applications. *Biomaterials*, vol.23(4): 1169–1185. http://dx.doi.org/10.1016/S0142-9612(01)00232-0
87. Hutmacher D W, Schantz T, Zein I, *et al*., Mechanical properties and cell cultural response of polycaprolactone scaffolds designed and fabricated via fused deposition modelling. *Journal of Biomedical Materials Research*, vol.55(2): 203–216. http://dx.doi.org/10.1002/1097-4636%28200105%2955%3A2%3C203%3A%3AAID-JBM1007%3E3.0.CO%3B2-7
88. Cesarano J, Segalman R and Calvert P, 1998, Robocasting provides mold less fabrication from slurry deposition. *Ceramic Industry*, vol.148: 94–102.
89. Smay J E, Cesarano J and Lewis J A, 2002, Colloidal inks for directed assembly of 3-D periodic structures. *Langmuir*, vol.18(14): 5429–5437. http://dx.doi.org/10.1021/la0257135
90. Michna S, Wu W and Lewis J A, 2005, Concentrated hydroxyapatite inks for direct-write assembly of 3-D periodic scaffolds. *Biomaterials*, vol.26(28): 5632–5639. http://dx.doi.org/10.1016/j.biomaterials.2005.02.040
91. Barnes C P, Sell S A, Boland E D, *et al.*, 2007, Nanofiber technology: Designing the next generation of tissue engineering scaffolds. *Advanced in Drug Delivery Reviews*, vol.59(14): 1413–1433. http://dx.doi.org/10.1016/j.addr.2007.04.022
92. Perera F H, Martínez-Vázquez F J, Miranda P, *et al.*, 2010, Clarifying the effect of sintering conditions on the microstructure and mechanical properties of beta-tricalcium phosphate. *Ceramics International*, vol.36(6): 1929–1935. http://dx.doi.org/10.1016/j.ceramint.2010.03.015
93. Yun H S, Kim S E and Hyeon Y T, 2007, Design and preparation of bioactive glasses with hierarchical pore networks. *Chemical Communications*, vol.21(21): 2139–2141. http://dx.doi.org/10.1039/B702103H
94. García, A, Izquierdo-Barba I, Colilla M, *et al.*, 2011, Preparation of 3-D scaffolds in the SiO_2–$P2O_5$ system with tailored hierarchical meso-macroporosity. *Acta Biomaterialia*, vol.7(3): 1265–1273. http://dx.doi.org/10.1016/j.actbio.2010.10.006
95. Sánchez-Salcedo S, Shruti S, Salinas A J, *et al*., 2014, *In vitro* antibacterial capacity and cytocompatibility of SiO_2–CaO–P_2O_5 meso-macroporous glass scaffolds enriched with ZnO. *Journal Materials Chemistry B*, vol.2(30): 4836–4847. http://dx.doi.org/10.1039/C4TB00403E
96. Martínez-Vázquez F J, Cabañas M V, Paris J L, *et al.*, 2015, Fabrication of novel Si-doped hydroxyapatite/

gelatine scaffolds by rapid prototyping for drug delivery and bone regeneration. *Acta Biomaterialia*, vol.15: 200–209.
http://dx.doi.org/0.1016/j.actbio.2014.12.021

97. Shruti S, Salinas A J, Lusvardi G, *et al*., 2013, Mesoporous bioactive scaffolds prepared with cerium-, gallium- and zinc-containing glasses. *Acta Biomaterialia*, vol.9(1): 4836–4844.
http://dx.doi.org/10.1016/j.actbio.2012.09.024
98. Cicuéndez M, Malmsten M, Doadrio J C, *et al.*, 2014, Tailoring hierarchical meso–macroporous 3D scaffolds: From nano to macro. *Journal of Materials Chemistry B*, vol.2(1): 49–58.
http://dx.doi.org/10.1039/C3TB21307B
99. Meseguer-Olmo, L, Vicente-Ortega V, Alcaraz-Baños M, *et al*., 2013, *In-vivo* behavior of Si-Hydroxyapatite/polycaprolactone/DMB scaffolds fabricated by 3D printing. *Journal of Biomedical Materials Research A*, vol.101A(7): 2038–2048.
http://dx.doi.org/10.1002/jbm.a.34511
100. Riza S H, Masood S H and Wen C, 2014, Laser-assisted additive manufacturing for metallic biomedical scaffolds, *Comprehensive Materials Processing*, vol.10: 285–301.
http://doi.org/10.1016/B978-0-08-096532-1.01017-7
101. Berry E, Brown J M, Connell M, *et al*., 1997, Preliminary experience with medical applications of rapid prototyping by selective laser sintering. *Medical Engineering & Physics*, vol.19(1): 90–96.
http://dx.doi.org/10.1016/S1350-4533(96)00039-2
102. Kruth J-P, Mercelis P, Vaerenbergh J V, *et al*., 2005, Binding mechanisms in selective laser sintering and selective laser melting. *Rapid Prototyping Journal*, vol.11(1): 26–36.
http://dx.doi.org/10.1108/13552540510573365
103. Wiria F E, Leong K F, Chua C K, *et al.*, 2007, Poly-Ɛ-caprolactone/hydroxyapatite for tissue engineering scaffold fabrication via selective laser sintering. *Acta Biomaterialia*, vol.3(1): 1–12.
http://dx.doi.org/10.1016/j.actbio.2006.07.008
104. Tan K H, Chua C K, Leong K F, *et al*., 2005, Selective laser sintering of biocompatible polymers for applications in tissue engineering. *Biomedical Materials Engineering*, vol.15(1–2): 113−124.
105. Shuai C, Li P, Liu J, *et al.*, 2013, Optimization of TCP/HAP ratio for better properties of calcium phosphate scaffold via selective laser sintering. *Materials Characterization*, vol.77: 23–31.
http://dx.doi.org/10.1016/j.matchar.2012.12.009
106. Lin C Y, Wirtz T, LaMarca F, *et al.*, 2007, Structural and mechanical evaluation of a topology optimized titanium interbody fusion cage fabricated by selective laser melting process. *Journal of Biomedical Material Research*, vol.83A(2): 272–279.
http://dx.doi.org/10.1002/jbm.a.31231